PREVALENCE AND PATHOLOGY OF NECROSIS OF EXTREMITIES IN CATTLE

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled 'PREVALENCE AND PATHOLOGY OF NECROSIS OF EXTREMITIES IN CATTLE' is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Name of the candidate : C.J. Xavier

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17-4-1989

CERTIFICATE

Certified that this thesis entitled 'PREVALENCE AND PATHOLOGY OF NECROSIS OF EXTREMITIES IN CATTLE' is a record of research work done independently by Sri. C.J. Xavier under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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To
Our Father in Heaven
for
His everlasting faithfulness

"The Lord shall make thee the head and not the tail..."
Deuteronomy 28 : 13

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Introduction

1. INTRODUCTION

A disease characterised by necrosis of the extremities was encountered in bovines particularly in buffaloes, in Kerala since the last three decades. The chief clinical manifestation of this disease is the necrosis of the tail. This condition is popularly known as 'Valucheeyal' in Malayalam and causes considerable economic loss to the farmers. The disease is identical in all respects to the 'Deg Nala' disease reported by earlier workers from other parts of the country and certain other parts of the world. It is believed that this disease syndrome is closely associated with the feeding of mouldy paddy straw to cattle. It has been pointed out that the 'Deg Nala' disease is prevalent only in the rice cultivating parts of the Indo-Pak region of the sub-continent. Since mouldy straw is incriminated in all these cases a mycotoxic aetiology was suggested (Kalra et al. 1977; Maqsood, 1984; Behera, 1985).

In Kerala, rice is the staple food of people and paddy straw constitutes the major bulk of the roughage ration of cattle. The post-harvest storage practices are not satisfactory and the farmers stock their straw in their respective land holdings in open air. The prevailing high humidity and ambient temperature are quite congenial for the development of fungal organisms in various feed ingredients of the livestock. The unsatisfactory post-harvest practices adopted again favour fungal growth.

Soon after the rainy season the straw becomes infested with various fungi which are capable of producing metabolites, most of which are toxic in nature. Factors like moisture, relative humidity, temperature, composition of substrate, fungal strain etc. influence mould growth on a commodity and they also influence toxin production. Of the physical parameters involved in fungal growth, moisture content, temperature and relative humidity are important.

Kerala State lies between 8°18' and 12°48' North latitude and between 74°32' and 77°24' East longitude. There is no distinct winter season and there are two wet periods and a dry period (Nair, 1973). There is absence of a seasonal rhythm and little variation in day length. Mean ambient temperature is 27°C and temperature as high as 40°C is experienced during the months of March and April. Relative humidity is high throughout the year. This aspect of environmental food contamination due to mycotoxins, which is relatively high in this State because of the high humid climate, was a neglected field of study.

The underlying cause of necrosis of the extremities was not investigated, although a mycotoxic aetiology was suggested by certain workers. In the present study it has been envisaged to study the incidence and nature of the disease.

Review of Literature

2. REVIEW OF LITERATURE

2.1. Prevalence

The necrosis of the extremities in cattle in the Indian sub-continent was first reported by Shirlaw (1939). During the years 1920-1930 there were reports from the Sheikhpura and Muridke parts of the Degnala area of Punjab, on a peculiar disease of buffaloes that caused severe economic loss and Shirlaw was forced to undertake a special enquiry into the pathology and aetiology of the condition. It was ascertained that not only buffaloes but occasionally zebu cattle of all ages were affected. Since the disease was more prevalent in the Deg Nala area (Deg Nala = Deg river) it was termed Degnala disease. This peculiar condition was encountered in buffaloes and occasionally in white cattle of the low lying marshy tract of the land bordering Deg Nala. According to the natives the disease had existed for generations and Shirlaw (1939) provided a very apt description of the symptoms, lesions and histopathology of the disease.

Barakat et al. (1960) observed a chronic disease condition 'Arrada' characterised by the necrosis of tail, among the Egyptian buffaloes. It was not noticed in cattle even when they were kept under similar conditions. This disease was of economic importance since it caused an appreciable reduction in the price of the affected buffaloes.

George et al. (1970) reported necrosis of tail among bovines treated at the Veterinary College Hospital, Mannuthy.

South India. Widespread outbreaks of necrosis of tail in cattle was noticed during 1968-69 in various parts of Kerala. Out of the 1,260 bovines admitted at the hospital during the period from September 1968 to May 1969, 142 were affected with tail necrosis, thus showing an overall incidence of 11.3 per cent. Of these, 920 were white cattle of which 52 were affected, i.e., 5.6 per cent and 340 were black cattle of which 90 were affected i.e., 26.5 per cent. The percentage of incidence was 50.8 in he-buffaloes, 20.2 in she-buffaloes, 4.3 in buffalo calves, whereas the percentage of incidence was only 17.8 in buffaloes, 1.4 in cows and 1.5 in calves. The percentage of incidence was greater in black cattle than in white cattle and in working animals as compared with farm livestock. The incidence of the disease was on the increase during January to April, the peak month being February and March and the percentage of incidence being 27 and 26 respectively.

From Pakistan, Irfan (1971) reported the clinical picture and pathology of the necrosis of extremities in buffaloes and cattle. He observed a seasonal incidence for this condition and stated that in 1968, 1969 and 1970 outbreaks occurred during November-February each year. Clinical pictures and histopathological changes were studied in 295 buffaloes and 10 white cattle. The age of these animals varied from three months to ten years. Both sexes were involved. The disease was sporadic in nature with seasonal incidence and was

prevalent during the winter months when rice crops were fully grown and the animals were fed on green rice fodder or fresh rice straw. The disease was confined to buffaloes and cattle but the latter showed only mild lesions. Irfan (1971) stated that this disease was no longer confined to the areas around Deg Nala, but was also prevalent in other low lying regions where rice was cultivated. In this investigation the author examined 25 herds in 15 different villages and 295 buffaloes and 10 white cattle were found affected.

Kwatra and Singh (1972) observed a similar disease condition from several villages of the district of Amritsar and a village each in districts of Hoshiarpur, Jullundur and Gurdaspur. Overlooking Irfan's (1971) publication, they redescribed the Deg Nala disease as a separate entity 'Gangrenous syndrome'. They associated its occurrence with winter and feeding of paddy straw.

Dhillon (1973) confirmed that the disease appeared about the same period, i.e., in 1968, 1969, 1970 in several villages of some of the rice growing districts, Gurdaspur and Amritsar, of the Punjab and in the villages around Karnal in Haryana. It was found that it occurred during the colder months when the animals were liberally fed on paddy straw.

Kalra et al. (1974) described the epizootiological observations, clinical features and pathology of an obscure disease commonly known as Degnala disease. Their findings were based on the investigations of disease outbreaks which

occurred during the years 1968 to 1972, in the rice growing areas of the Haryana State. The disease, having chronic ergot poisoning like syndrome, was sporadic, seasonal and regional in character with a tendency to confine itself to a particular herd or field. The morbidity rates in buffaloes and cattle were 67.9 per cent and 13.61 per cent respectively. The mortality rate was 22.21 per cent in buffaloes and 3.27 per cent in cattle.

Rajan et al. (1977) reported on the prevalence and pathology of the necrosis of the extremities in bovines of Kerala State. They pointed out the resemblance of this condition to Degnala disease. Buffaloes were primarily involved and the disease had a seasonal incidence.

Said et al. (1977) collected one hundred specimens of tail necrosis from a slaughter house at Cairo, Egypt. They also examined 12 clinical cases.

Martig and Leuenberger (1978) reported necrosis of tail in a bull fattening unit in Switzerland. Out of 128 animals, 119 had tail lesions, 49 of these had lost part of their tail.

From China observation on the necrosis of extremities in cattle was reported. A disease characterized by the necrosis of ears, tail and feet was observed by Deng et al. (1984) in Guilin district, Guangxi, during the period 1979-83. They found that the mortality rate was 35-43 per cent and the animals had consumed mouldy rice straw.

While conducting comparative study on urea enriched wheat/paddy straw rations Bakshi et al. (1986) observed symptoms described for Degnala disease in Buffaloes. They made some investigations to find out the causative factor responsible for the development of this disease in buffaloes on feeding rice straw.

2.2. Aetiology

Shirlaw (1939) was the first to make attempts to throw light into the aetiological aspects of the necrosis of extremities, which the villagers of the Deg Nala area termed 'Leprosy' because of its superficial appearance. Being one of the largest grazing areas in Punjab, most of the epizootic diseases periodically made their appearance. It was a fertile ground for the breeding of parasitic diseases, while the rank coarse herbage afforded ideal condition for the propagation of insect vectors of disease especially of Tabanus and Ticks (Shirlaw, 1939). During post-mortem examination Shirlaw observed Schistosomes of the species S. indica in the liver of affected animals. The parasitic atheroma encountered in the aorta was attributed to Onchocerca armillata and Eleophora poeli (Shirlaw, 1939). Shirlaw also considered the probability of ingestion of poisonous plants or fodder. But no evidence was obtained from the direct examination of fodder. During the course of investigation he examined the possibility of ergotism, protozoal, bacterial and helminthic infections, but could not specify the exact cause of the disease.

El-Mekkawi (1958) claimed that of the various organisms isolated Corynebacterium bovis isolated from tails of sick buffaloes was the causative organism for the necrosis of tail in Egyptian buffaloes. He also stated that an effective method of treatment was to dip the affected tail in 10 per cent formalin.

Barakat et al. (1960) suggested that the necrosis of tail in Egyptian buffaloes was of the nature of a deficiency disease. An emulsion consisting of egg-yolk and linseed oil was recommended for the treatment. The authors, therefore, concluded that deficiency of fatty acids was responsible for this syndrome.

Irfan (1971) was able to relate the feeding of green rice fodder/fresh paddy straw with Degnala disease. He had noticed that this disease had spread to other low-lying areas, other Degnala, where rice was cultivated. Though, he initially suspected ergot poisoning, no natural cases of ergot poisoning were detected from these areas.

Kwatra and Singh (1972) and Dhillon (1973) suggested the association of this syndrome with the feeding of paddy straw during winter months.

Arora et al. (1975) reported that selenium toxicity caused by feeding selenium rich paddy straw could be the main cause for the development of this disease in buffaloes. They observed that the selenium levels of fodder samples obtained from the owners of sick animals ranged between

0.9 ppm to 6.7 ppm which were above the safer level of 0.5 ppm.

Arora (1977) gave further association of the Degnala disease with selenium toxicity. He observed that, the animals suffering from the disease had recovered fully when they were treated with 'Degcure' an antidote mixture prepared in the Nutrition Division of National Dairy Research Institute, Karnal, at the rate of 30 g per day per animal.

Kalra et al. (1977) suggested that paddy straw contaminated with toxic fungi were the aetiological factors for Degnala disease. They indicated the Fusarium mycotoxins as a possible cause for this condition.

Said et al. (1977) observed Microfilariae in the blood samples of animals suffering from tail necrosis in Egypt. These animals also gave a positive reaction to filarial antigens. Treatment with antifilarial drugs stopped the progress of the lesions.

Martig and Leuenberger (1978) attributed intensive nature of housing and nutrition for the necrosis of tail in a bull fattening unit in Switzerland.

Bhatia and Kalra (1981) reported that, Degnala disease could be experimentally produced in buffalo calves by feeding rice straw which was suspected to be contaminated with mycotoxins.

Prasad et al. (1982) found that feeding of rice husk containing 6.23 ppm selenium can also cause this disease

syndrome. They were able to induce selenium toxicity. Also it was observed that those animals treated with 'Degcure' (formula - Magnesium sulphate - 1 kg; ferrous sulphate - 16 g; copper sulphate - 24 g; zinc sulphate - 75 g; cobalt sulphate 15 g) recovered from the disease.

From China Deng et al. (1984) attributed the consumption of mouldy rice straw as the possible cause for the necrosis of extremities in cattle. They isolated F. equiseti, F. semtectum and F. anquioides from mouldy rice straw.

Maqsood (1984) indicated the possible role of F. equiseti for causing the Degnala disease in buffaloes and cattle of the Indian sub-continent. The animals succumbed to this syndrome by consuming the fungus infested rice straw.

Behera (1985) reported that mycotoxins belonging to trichotheene group in the fungus infested rice straw were responsible for Degnala disease in buffaloes.

Zhang et al. (1985) reported that eight species of Fusarium were isolated from the mouldy rice straw from three regions in Guizhou province of China, where foot lesions were common among cattle. F. sporotrichoides and F. equiseti were the common isolates.

Bhatia and Gupta (1986) observed that the rice straw from the area where the disease had occurred yielded known mycotoxin producing fungi, the most numerous being F. equiseti. They produced the disease in buffalo calves by feeding contaminated paddy straw. They also observed that treatment with

'Degcure' had no effects so long as the contaminated rice straw was fed.

Bakshi et al. (1986) observed that Degnala disease like symptoms appeared in buffaloes in areas where there is increased selenium uptake by rice plants.

2.3. Clinical picture and pathology

Shirlaw (1939) observed the first incidence of the disease with the clinical features of general malaise unaccompanied by fever and attended by signs of vague abdominal pain, disinclination for movement and a painful gait suggestive of laminitis. Anorexia was present though animals showed a desire for food. Examination of the tongue revealed a marked anterior swelling with considerable pain on manipulation. After a variable period of time, areas of subcutaneous oedema developed, generally confined to the extremities, i.e., feet, tail and ears or occasionally more widespread in the posterior abdominal area and perineum. Shirlaw (1939) noticed in one or two cases swelling of the entire head region. Gradually the swellings abated, thereby rendering more definitive the exact location of the lesion which, on palpation appeared less oedematous and more proliferative. At this stage the initial malaise disappeared, the affected animal started feeding normally and apart from a slight lameness and swelling of the feet, the majority of animals returned to normal health. However, Shirlaw (1939) observed that 20-30 per cent of animals did not show the termination. Such cases remained

decidedly ill. Rapid loss of the condition and anaemia with marked eosinophilia were seen. A secondary oedema was seen localised in the proximity of the dewlap, while the oedema first noted in the extremities increased in volume, the overlying skin became tense, devoid of sensation. The oedematous fluid was gradually absorbed and replaced by a proliferative tissue which, produced in excess, conferred a marked thickening of the affected part. The overlying skin became dry and rough in texture and necrotic in appearance and quite insensitive. Neither the lymph vessels nor the superficial lymph-nodes were affected. The proliferative induration of the subcutis and the dry wrinkled skin accorded an elephantiasis like appearance to the affected limbs. The ears and tail were affected with dry gangrene and a sharp line of demarcation was seen between the dead and healthy tissues. Sloughing shortly occurred and the animals lost their ears and tail. Not infrequently similar fate befell the anterior portion of the tongue.

The affected areas of the skin of the limbs fissured deeply and the necrotic skin peeled away from the underlying tissues, exposing a bleeding raw surface which normally underwent cicatrisation in spite of its liability to secondary infection. Lesions around hoof were more serious. Necrotic ungulitis resulting in shedding of digits occurred. Occasionally the entire hoof was lost. Animals exhibited marked prostration and cachexia.

Shirlaw (1939) recorded the post-mortem examination findings of eleven animals dead or destroyed in various stages of the disease. On dissection of an affected limb the subcutis was found to be indurated by new connective tissue. Histological examination showed mild degree of mononuclear infiltration and a certain proportion of eosinophils. Connective tissue proliferation was observed. There was no tendency to focal necrosis or aggregation of leucocytes with vascular congestion and secondary inflammatory changes as usually encountered in an infective process. The oedematous fluid distended the subcutaneous tissues along the channels of least resistance. The blood vessels of the new tissue showed various stages of a process culminating in total occlusion. There was no thrombosis, but a progressive mesarteritis resulting in gross thickening of the vessel wall and constriction and final obliteration of the lumen was prominent. Synchronous with this process, formation of new arterial collateral supply was also observed. But they also underwent the pathological changes affecting the parent vessels. The veins were found to be dilated to the maximum capacity. The vessels, both arteries and veins appeared as thickened cords. Parasitic atheroma was encountered in nine out of eleven cases (Shirlaw, 1939).

El-Mekkawi (1958) described the pathological changes in necrosis of tail in Egyptian buffaloes. It consisted of marked hyperkeratosis of the epidermis. He observed consolidation and homogenisation of the connective tissue together with collapse of blood vessels.

Barakat et al. (1960) reported that the tail necrosis in Egyptian buffaloes had its onset marked by loss of hair at the tip of the tail followed by hyperkeratosis, later culminating in necrosis. If left untreated, the necrosis extended slowly upwards to the base of the tail and finally the buffalo was deprived of its tail. The disease was not transmitted when necrotic and healthy buffaloes were kept in contact with each other. The necrosed tail usually showed eruptions on the surface.

George et al. (1970) provided a vivid clinical picture of the necrosis of extremities of bovines in Kerala. The lesion was seen at the tip of the tail, with typical inflammatory symptoms, which gradually progressed upwards. The affected region was slightly swollen at the onset. It later became insensitive and cold to touch and the hairs began to fall off. This stage usually passed unnoticed owing to the presence of the switch. The lesion extended proximally and a line of demarcation was seen between the distal necrosed and the proximal healthy portion. It became a dry gangrene. The affected region became hard, dry and thin and sloughing took place at the line of demarcation. In some cases, suppuration took place, causing progressive moist gangrene, the extent of which was unpredictable. In a few cases, necrosis of the extremities like the tip of the ear, fetlock and brisket was also present. Dissection of the necrosed distal portion revealed the presence of a well organised clot in

the coccygeal vessels obliterating the blood supply. In few cases the clot extended to two to three centimeters into the apparently healthy proximal portion.

Irfan (1971) recorded the clinical picture and pathology of Degnala disease in buffaloes and cattle in West Pakistan. The first indication of the disease appeared when the animals went off feed for a few days. During the early stages of the disease some animals showed lachrymation and dribbling of saliva. Within a few days, oedematous swellings appeared on the lower region of the legs and other dependent parts of the body. In many cases the legs were enormously thickened. As the disease progressed, ulcerated wounds appeared on the legs, tips of the tail and ears. Lesions ultimately led to necrosis and even gangrene. The affected parts were cold to touch. The hair on these affected parts denuded and a line of demarcation was seen between the diseased and healthy parts. In some cases the gangrenous portions sloughed off. Ulcerated wounds also appeared on the muzzle and tip of the tongue. In some cases these parts also sloughed off with the onset of gangrene. The animals became lame and some became prostrate due to pain from the wounds. They lost their condition. Usually the wounds healed in due course. The blood vessels in the affected areas were found to be thickened and hardened. In some cases the vessel walls were almost double the normal size, and their lumen was constricted. Extensive fibroblastic proliferation was observed in the subcutaneous region. Degenerative changes were observed in the muscle bundles. The tunica media of the blood vessels was thickened.

Kwatra and Singh (1973) reported that Degnala disease was characterised clinically by development of oedema, necrosis and gangrene of the extremities. Haematological values of the experimental cases were not found to vary from those of the normal animals. According to them the differential leucocyte count, total leucocyte count and packed cell volume did not show significant variation in the experimental cases. However, some animals showed slight transient fall in haemoglobin content.

Kalra et al. (1974) described the early symptom as disinclination on the part of the animal to move. This was followed by the appearance of oedematous swelling on the lower region of the legs and other dependent parts of the body. With the involvement of the legs, the animal developed lameness. As the disease progressed, necrosis and gangrene of the extremities of the legs, tail, ears, etc. developed. In a few cases, muzzle, lower jaw and tongue were also involved. The gangrenous areas were cold and insensitive to pressure or pricks. Later, there was sloughing of the affected parts resulting in ulceration and wound formation. In severely affected cases, the hooves were shed off, exposing the sensitive laminae and underlying bones. Affected animals could not move and remained recumbent. They became weak, emaciated and revealed hide bound condition. The dissection of gangrenous parts revealed necrosed tissues with thick walled arteries. The lumen of the vessels was reduced and

some of them showed thrombus formation. There was gelatinous degeneration of body fat. No significant lesion could be found in the visceral organs. Histopathological examination revealed proliferation of muscle fibres of the tunica media. There was slight to marked eosinophilic infiltration in the subcutaneous connective tissue, particularly around the smaller blood vessels.

Rajan et al. (1977) recorded the gross lesions and detailed histopathology of the necrosis of extremities in bovines in Kerala State. The affected animals were very weak and emaciated. The tail was thin, dry, shrivelled and cold to touch. The skin of the tail was wrinkled, rough and parchment like with scaly material coating the surface. The skin was only sparsely coated with hair. Tail was rigid and inelastic. Cut surface showed atrophic muscles and prominent coccygeal vessels filled with dark masses of blood clot firmly adherent to the vessel wall. Dissected out vessels were rigid and inelastic with reduced lumen. The carcases of the buffaloes that were autopsied were very much emaciated. Muscular tissue and skin over the anterior third of the maxilla and mandible had sloughed off exposing proximal part of these bones. Hooves of the limbs were shed off exposing the sensitive laminae and bony tissue. Lower portion of the limbs were diffusely swollen and the skin had peeled off exposing the muscular tissue. Muscular tissue of the exposed portion was diffusely inflamed. The prefemoral and prescapular

lymphnodes on both sides were enlarged and oedematous. Hydropericardium and ascites were observed in moderate degree. Subcutaneous tissue showed oedema and gelatinisation of fat.

Liver showed focal areas of fatty change and centrilobular necrosis. Kidneys revealed tubular degeneration and necrosis. In the spleen depletion of lymphoid tissue and moderate haemosiderosis were observed. Myocardium revealed focal areas of hyaline degeneration. Capsule of the adrenal glands was thickened. Focal areas of haemorrhage were observed at the cortico-medullary junction. Zona fasciculata was devoid of fat.

Tissues from the tail revealed focal areas of purulent dermatitis and trichofolliculitis characterized by neutrophilic and eosinophilic infiltration. Hyperkeratosis, parakeratosis and moderate degree of acanthosis were observed. Blood vessels showed pronounced thickening of the wall, reduction in the size of the lumen and hypertrophy of the tunica media.

Many of the blood vessels revealed thrombi. Muscular tissue had undergone degeneration and necrosis. Degeneration and rarefaction of the bony skeleton of the tail were evident. The coccygeal nerves did not reveal any evidence of degeneration except in advanced cases of gangrene of the terminal part of the tail.

Bhatia and Kalra (1981) conducted clinico-haematological

studies on Degnala disease. The earliest signs were disinclination to move and swelling of the lower parts of the leg particularly adjoining fetlock region resulting in lameness. Gradually there was loss of appetite and the animal became weak and emaciated. The affected parts were (legs, tail and ears) painful to touch. Subsequently necrosis followed. The necrotic portions became gangrenous and were cold and insensitive to pressure or pricks. Later the gangrenous parts sloughed off and gave rise to ulceration and wound formation. The animals showed hide-bound appearance and remained recumbent till death. During the experimental production of the disease, by feeding rice straw, it was not possible to line out any set pattern of the sequential involvement of different parts of the body. However, in general, the legs were the first to get involved, later tail and ears. In majority of the cases the hind legs, more often than the forelegs were involved. The clinical signs appeared first in the right hind leg. In experimental animals only slight gradual fall in leucocyte and haemoglobin values were observed. Reduction in eosinophils was observed in the later phase of the disease. But in natural cases a mild leucocytosis was noticed.

Bakshi et al. (1986) reported the experimental production of Degnala disease like syndrome in buffaloes, while conducting comparative studies on urea enriched wheat/rice straw rations. In buffaloes fed with rice straw initial symptoms of swollen extremities followed by necrosed skin

spots below the hock joints were observed within 30 days. The necrosis on tail and ear tips had also started. At a later stage the desquamation of the necrosed skin spots and skin around the hooves had resulted in the separation of hoof from the laminae resulting in the sloughing of hooves. The animals showed alopecia, staggering gait and general debility. At the terminal stage the animal became prostrate with stretched limbs.

Materials and Methods

3. MATERIALS AND METHODS

The study on the prevalence and pathology of Necrosis of the Extremities in cattle was conducted at the Centre of Excellence in Pathology, Mannuthy in liaison with the University Veterinary Hospitals and other peripheral veterinary institutions of the State Animal Husbandry Department in various districts.

3.1. Prevalence

In order to assess the prevalence of the condition the data published in the Disease Surveillance Report of the Animal Husbandry Department, for the periods 1985, 1986 and 1987 were utilized.

3.2. History

The modus operandi was to intimate the veterinarians all over the State through circular regarding the significance of this research programme. Feed back responses were expected with regard to the incidence of the disease. On receiving information arrangements were made to undertake an on-the-spot study of the cases including the clinical examination of the animal/animals, history and collection of specimens.

3.3. Haematological studies

Blood samples from the animals were collected from the jugular vein with and without anticoagulants. Procedures described by Schalm (1975) were followed for the determination

of erythrocyte sedimentation rate, packed cell volume, haemoglobin, total and differential leukocyte counts and total erythrocyte count.

3.4. Estimation of Calcium and Magnesium in the serum

The levels of calcium and magnesium in the sera were estimated using the atomic absorption spectrophotometer method. Procedure given by the Manual of Perkin Elmer Company, U.S.A. was adopted.

3.5. Estimation of inorganic phosphates in serum

Inorganic phosphate in the serum was estimated by the Fiske and Subba Row method as described by Oser (1979).

3.6. Estimation of total Plasma Protein

Serum protein was estimated by the Biuret Assay method of Inchiosa (1964).

3.7. Mycological studies

Mycological studies were undertaken to find out the role of pathogenic fungi in the causation of necrosis of extremities. Suspected straw samples were collected at random from farmers whose animals had the disease syndrome and were cultured for isolation of fungi. Processed samples were inoculated into Sabouraud's Dextrose Agar for the isolation of fungi (Emmons et al. 1977). Plates were incubated at room temperature for a maximum period of 21 days. The fungal

growths discernible macroscopically during this period were further identified by clinical and morphological characteristics. Morphological characteristics were studied by tease mount preparation using lactophenol cotton blue as a mounting medium (Larone, 1976). The growth and sporulation of fungi in an almost undisturbed condition were studied using Riddles slide culture technique for further identification (Al-Doory, 1980; Choudhari and Chandrashekhara, 1981).

3.8. Paddy straw extraction and Thin Layer Chromatographic studies

The paddy straw collected from the disease prevalent areas were analysed for trichothecenes. Fourteen samples of suspected paddy straw and four samples of apparently good straw were extracted (for 4 to 6 hours) with 1 litre of ethylacetate. The extract was washed with 50 ml of distilled water and evaporated on a water bath for dryness. It was dissolved in 200 ml of chloroform:methanol mixture (4:1) and shaken with 80 ml hexane. The upper hexane layer was discarded and 60 ml of distilled water added. It was extracted again using 100 ml of chloroform:ethylacetate mixture (1:1). The lower layer was taken and evaporated. This rice straw extract was dissolved in 2 ml of ethylacetate. A small (0.1 ml) aliquot from each sample was applied on Thin Layer chromatography plate and the plate was developed in chloroform-methanol (97:3) mixture. The plate was dried and sprayed with 20 per cent methanolic sulfuric acid (20 ml H_2SO_4:80 ml methanol).

The plate was later kept at 110°C for 10 minutes and examined under long wave length U.V. light.

3.9. Radiographic studies

Contrast radiography was employed to study the *in vivo* profile of arteries and veins in the necrosed tail of a buffalo. The coccygeal artery was exposed under local anaesthesia and the contrast medium Conray-420 (M & B) was injected. X-ray was taken to get the angiogram and venogram of the tail. Similarly angiogram and venogram of two normal tails were also taken.

3.10. Gross Pathology

The affected tails were examined in detail and gross lesions were recorded. Representative samples of tissues from the affected tails were taken and fixed in 10% buffered formalin.

3.11. Histopathology

The formalin fixed tissues were subjected to decalcification in 5 per cent nitric acid (Luna, 1968). Decalcified tissues were processed by routine methods (Luna, 1968; Sheehan and Harapchak, 1980). Paraffin sections cut at 3-5 u thickness were stained with Harris' Haematoxylin-Eosin, Van Gieson's stain and Verhoeff's stain.

4. RESULTS

4.1. Prevalence

According to the livestock census of Kerala (1982) there are 3096775 white cattle and 408584 buffaloes (Table 1). The incidence of the disease syndrome was found more in buffaloes than in white cattle. In 1985 the incidence in white cattle was 0.0175% and in buffaloes it was 0.1343%. In 1986 it was 0.0155% and 0.1534% respectively. During the first half of 1987 the incidence was 0.0124% and 0.07709% respectively (Table 2).

4.2. History

During the field visits veterinarians reported that cases were presented at the hospitals stating that there was falling of hair on the tail. In the present study, in all the cases examined the lesion was restricted to the lower extremity of the tail. The veterinarians observed that in certain instances animals were brought to the hospitals with the tail sloughed off. Also they reported that, if left untreated the necrosis progressed upwards. However, in certain cases, the condition culminated as a self-limiting necrosis. The affected animals were feeding and drinking normally. They appeared alert and active without any apparent systemic involvement.

All the affected animals were kept close to the house of the farmers. Housing conditions were in general satisfactory.

Table 1. Livestock population in Kerala*

	Cattle		Buffaloes	
	Number	Percentage	Number	Percentage
Trivandrum	197501	6.38	31438	7.69
Quilon	288924	9.33	18148	4.44
Pathanamthitta	235053	7.59	8790	2.15
Alleppey	214542	6.93	6826	1.67
Idukki	166028	5.36	14193	3.47
Kottayam	283353	9.15	7931	1.94
Ernakulam	304367	9.83	27272	6.67
Trichur	233655	7.55	54331	13.30
Palghat	273813	8.84	102354	25.05
Malappuram	193364	6.24	64428	15.77
Calicut	212568	6.86	7200	1.76
Wynad	108964	3.52	28642	7.01
Cannanore	223124	7.21	13440	3.29
Kasargod	161519	5.22	23591	5.77
Total	3096775		408584	

* Livestock census, Kerala, 1982,
Directorate of Animal Husbandry, Trivandrum.

Table 2. Prevalence of Tail Necrosis

	1985		1986		1987	
	Cattle	Buffaloes	Cattle	Buffaloes	Cattle	Buffaloes
January	53	41	35	24	32	35
February	33	62	42	66	139	44
March	35	55	36	106	30	32
April	79	43	44	66	38	48
May	21	28	48	52	58	43
June	27	42	33	53	57	67
July	23	39	37	40	-	-
August	144	75	50	40	-	-
September	31	33	47	60	-	-
October	31	47	37	42	-	-
November	27	40	37	42	-	-
December	34	44	35	36	-	-
Total (number)	545	549	481	627	387	315
Prevalence in percentage	0.0175	0.1343	0.0155	0.1534	0.0124	0.07709

All the animals in a particular household were not affected. However, in one instance, all the four buffaloes which were kept in a single shed were affected. In another place only two buffaloes among four kept in a shed were found to be affected. In certain places animals which were purchased from the neighbouring State of Tamil Nadu, developed the condition. In these cases it was not clear whether these animals already had the disease in sub-clinical form when they were purchased or the lesion came to the notice of the farmer only after they were being reared in Kerala conditions.

During the field visits, it was observed that these animals were fed paddy straw purchased locally as the sole fodder. These farmers were not in a position to feed their livestock with paddy straw from a single source. Most of them were depending upon daily/weekly supply of straw purchased from straw traders. It was observed that the paddy straw which was being fed to the animals was obtained from different sources. In many cases, the straw had a mould contaminated appearance.

4.3. Haematological studies

The haematological parameters recorded from the clinical cases are given below and is represented in tables 3 and 4.

4.3.1. Haemoglobin (g/dl).

The mean haemoglobin value was 11.75 $\pm$ 0.56 g/dl.

Table 3. Haemogram of buffaloes with tail necrosis

Animal No.	Hb (g/dl)	PCV (%)	ESR (mm/hr)	RBC (10^6/ul)
1	11	31	57	5.2
2	14.5	42	55	7
3	13	38	60	6
4	10	32	65	5.5
5	10	30	61	5
6	12	37	55	6.2
7	14.5	43	56	7.2
8	12	30	60	5.1
9	10.5	35	58	5.9
10	10	34	57	6.1
Mean ± S.E.	11.75 ±0.56	35.20 ±1.50	58.4 ±1.0	5.92 ±0.24

Table 4. Differential count of buffaloes with necrosis of tail

Animal No.	Eosino-phils	Neutro-phils	Lympho-cytes	Monocytes
1	4	34	58	4
2	3	35	60	2
3	1	30	66	3
4	3	45	50	2
5	3	31	54	2
6	2	40	56	2
7	3	37	58	2
8	2	42	54	2
9	3	34	60	3
10	2	34	62	2
Mean ± S.E.	2.6 ±0.23	36.20 ±1.52	57.80 ±1.44	2.40 ±0.2211

4.3.2. Packed Cell Volume.

The mean packed cell volume was 35.20 ± 1.50 per cent.

4.3.3. Erythrocyte Sedimentation Rate.

The mean erythrocyte sedimentation rate was 58.40 ± 1.0 mm/hr.

4.3.4. Total Erythrocyte Count (10^6/ul).

The mean total erythrocyte count was 5.92 ± 0.24 million/ul.

4.3.5. Differential leucocyte count.

4.3.5.1. Eosinophils.

The mean eosinophil count was 2.60 ± 0.23.

4.3.5.2. Neutrophils.

The mean neutrophil count was 36.20 ± 1.52.

4.3.5.3. Lymphocytes.

The mean lymphocyte count was 57.80 ± 1.44.

4.3.5.4. Monocytes.

The mean monocyte count was 2.40 ± 0.22

4.4. Calcium and Magnesium level in serum

4.4.1. Calcium.

The mean calcium level in serum was 8.82 ± 0.18 mg % (Table 5).

4.4.2. Magnesium.

The mean Magnesium level in serum was 2.57 ± 0.11 mg % (Table 5).

Table 5. Calcium, Magnesium and inorganic phosphate level in serum of buffaloes with tail necrosis (mg %)

Animal No.	Calcium (19)	Magnesium (19)	Inorganic phosphates (10)
1	9.60	3.18	6.0
2	9.26	3.53	7.0
3	8.63	2.10	6.2
4	8.19	2.64	7.6
5	9.33	2.95	5.5
6	8.93	2.21	6.9
7	7.55	2.82	6.0
8	10.34	2.77	7.0
9	8.94	2.06	7.2
10	9.64	2.42	7.0
11	9.06	2.00	-
12	8.26	2.34	-
13	8.76	3.19	-
14	9.42	2.08	-
15	8.63	2.91	-
16	7.53	2.70	-
17	7.38	2.43	-
18	8.92	2.07	-
19	9.20	2.46	-
Mean ± S.E.	8.82 ±0.18	2.57 ±0.11	6.64 ±0.21

Figures in parenthesis indicate the number of animals studied

4.5. Inorganic phosphates in serum

The mean inorganic phosphate in serum was 6.64 ± 0.21 mg% (Table 5).

4.6. Total plasma proteins

The mean total plasma protein level was 7.30 ± 0.80 g % (Table 6).

4.7. Mycological studies

The suspected paddy straw collected from various locations were subjected to cultural examination, to identify the nature of fungi contaminating the straw. Also apparently clean paddy straw samples were subjected to examination. The results are shown in the table 7.

4.8. Thin Layer Chromatographic studies

Paddy straw extracts obtained from field samples were subjected to Thin Layer Chromatographic Studies. Trichothecenes could not be detected during the present study.

4.9. Radiographic Studies

Radiographic studies of the normal and diseased tails were undertaken. Also artereograms and venograms of the animals were taken (Fig.1).

Table 6. Total plasma protein level in buffaloes with tail necrosis

Animal No.	Total plasma protein (g %)
1	7.5
2	7.0
3	7.3
4	6.9
5	7.1
6	6.9
7	7.7
8	7.9
9	7.2
10	7.5
Mean ± S.E.	7.30 ± 0.11

Table 7. Mycological studies on suspected samples of paddy straw

Place	District	Genus of the fungus
1. Amballore	Trichur	
Sample I		Penicillium, Mucour, Aspergillus
Sample II		Penicillium, Aspergillus, Rhizopus
2. Angamaly	Ernakulam	Aspergillus, Penicillium, Rhizopus, Nigrospora
3. Anthikkadu	Trichur	Penicillium, Aspergillus
4. Chalissery	Trichur	Aspergillus, Mucour
5. Kalladikkode	Palghat	
Sample I		Mucour, Rhizopus, Nigrospora
Sample II		Curvularia, Penicillium, Aspergillus
Sample III		Mucour, Penicillium, Rhizopus
6. Kunnamkulam	Trichur	Aspergillus, Penicillium Rhizopus
7. Kuthannore	Palghat	Mucour, Rhizopus
8. Malampuzha	Palghat	Penicillium, Nigrospora, Rhizopus
9. Mannuthy	Trichur	
Sample I		Aspergillus, Rhizopus, Mucour
Sample II		Penicillium, Rhizopus, Alternaria
10. Pattambi	Palghat	Curvularia, Nigrospora, Penicillium
11. Kokkalai	Trichur	Alternaria, Rhizopus, Mucour
12. Sreekrishna-puram	Palghat	Nigrospora, Aspergillus, Rhizopus
13. Vadakkancherry	Palghat	Penicillium, Mucour, Rhizopus

(contd.)

Table 7 contd.

Place	District	Genus of the fungus
14. Wadakkancherry	Trichur	
Sample I		Rhizopus, Aspergillus, Penicillium
Sample II		Aspergillus, Penicillium, Mucour
Sample III		Alternaria, Rhizopus, Mucour
Sample IV		Aspergillus, Penicillium, Curvularia
Mycological studies on apparently clean paddy straw		
1. Kalladikkode	Palghat	Aspergillus, Penicillium, Mucour
2. Pattambi	Palghat	Aspergillus, Rhizopus, Penicillium
3. Mannuthy	Trichur	
Sample I		Penicillium, Rhizopus
Sample II		Aspergillus, Mucour

4.9.1. Arteriograms of the normal tail.

The middle coccygeal artery with its branches were seen. The branches from the artery originated in level with the body of the coccygeal vertebrae on either side and anastomosed supplying the segments of the tail. The artery was continuous upto the distal end of the tail (Fig.1, 2).

4.9.2. Plain radiograph of the diseased tail.

An increase in the density of the soft tissue at the zone of necrosis with lytic changes of the coccygeal vertebra at the level of necrosis. The tissue with increased density covered the vertebra like 'cone' and the lytic changes of vertebra was noticed at the point where the 'cone' like soft tissue mass terminated. The vertebra distal to the zone of lysis had a reduced density (Fig.3).

4.9.3. Arteriogram of the diseased tail.

The middle coccygeal artery terminated in level with the vertebra preceding the one which had undergone lysis. A large number of tortous arterial branches originate from the artery and went into the cone shaped dense mass. Anastomotic branches also terminated in level with the region where the vertebrae had undergone lysis. The lateral coccygeal veins originated from the region where the artery had terminated. Arteriovenous connection was also very much appreciable (Fig.5).

4.9.4. Venogram of the diseased tail.

The lateral coccygeal veins originated from the level

of the diseased vertebra. The veins had the origin in the dense tissue that surrounded the vertebra (Fig. 4).

4.10. Gross Pathology

The gross lesions encountered in the tail were characteristic of dry gangrene. It started from the tip of the tail and extended upwards. No signs of moist gangrene were seen in observed cases. The affected tails were dry, insensitive and hairs were falling off (Fig. 6). Clear demarcation between affected distal portion and healthy proximal portion could be appreciated. Skin surface appeared wrinkled and was coated with scaly material. Cracks and fissures were visible on the skin. Below the line of demarcation the cut surface showed atrophic muscles and prominent coccygeal vessels were filled with dark masses of firmly adherent blood clot.

4.11. Histopathology

Systematic histopathological studies were made on the affected tails. The primary lesions were vascular in nature.

4.11.1. Arteries.

Coccygeal arteries had thick wall and a narrow lumen. The tunica media was very much thickened and by Van Gieson stain this was identified to be due to marked hypertrophy of the muscle fibres of the tunica media (Fig. 7). The thickening of the musculature varied from moderate to severe degree and the size of the arterial lumen was also found varying

(Fig.8). Thrombosis was a consistent feature. Thrombi in varying stages of organisations were observed (Fig.9). In certain places thrombi was well organised and completely occluded the lumen of the vessel. Thrombi showed varying degree of organisation. In certain places canalisation was observed and the vascular channels lined with endothelium were seen (Fig.10). Sub-intimal thrombosis was also seen in certain places (Fig.11). The intimal layer was raised and bulged into the lumen. In certain blood vessels elastic tissue staining revealed fragmentation of the elastic fibres (Fig.12). Replacement fibrosis was also a feature due to the partial or total destructions of the elastic fibres (Fig.13). Vas Gieson's stain demonstrated the collagenisation. Around the areas of thrombosis there were many new capillaries of small calibre indicating the formation of collateral circulation. Neo capillary proliferation was associated with granulation tissue formation (Fig.14). In certain places there was extensive proliferation of pericytes, particularly around the medium sized arteries (Fig.15). Mild to severe fibrosis was observed in certain cases, extending from the tunica externa to the tunica interna (Fig.16).

Several sections of each tail were examined but there was no indication of involvement of any parasites.

4.11.2. Veins.

The veins had an intact endothelial lining. There were no thrombi and the lumen was patent. There was only mild vascular sclerosis.

4.11.3. Nerve fibres.

The nerve fibres were seen scattered amidst a fibro-collagenous and degenerated mass of tissues (Fig.17). The size and distribution of the coccygeal nerves were found varying (Fig.18). Some were atrophic and appeared as small groups of nerve fibres. Intraneural and perineural fibrosis of varying degree were observed (Fig.19). No inflammatory signs were evident. The neurolemmal sheath was collapsed and the endoneurium and perineurium were intact.

4.11.4. Coccygeal vertebra.

There was indication of pronounced thinning of the osseous tissues. Osteolysis characterized by peripheral thinning of the vertebral osseous skeleton was evident (Fig.20). Surrounding the peripheral osteolytic zone of the osseous tissue there was fibrosis and collagenisation characterised by the presence of proliferating spindle shaped cells. Osteo-blastic activity was not evident. However, very mild osteo-clastic response was seen.

4.11.5. Skin.

Dermatitis of a chronic nature was a feature. Epidermis showed hyperkeratosis, parakeratosis and moderate to severe acanthosis (Fig.21). Proliferating prickle cells were seen dipping into the dermis forming large columns. There was, moderate to severe dermal fibrosis. Accessory glandular structures were cystic. The muscle tissues had undergone

extensive hyalinisation and fibrosis. Oedema and moderate to severe necrosis were seen (Fig.22). The hair follicles had undergone degeneration and most of them were devoid of hairs. Some of the follicles had undergone hyalinization (Fig.23), while cystic dilatation of the hair follicles was observed in some cases (Fig.24).

Fig. 1. Arteriogram of normal tail - Ventro dorsal aspect - showing the arterial supply

Fig. 2. Arteriogram of normal tail - lateral view

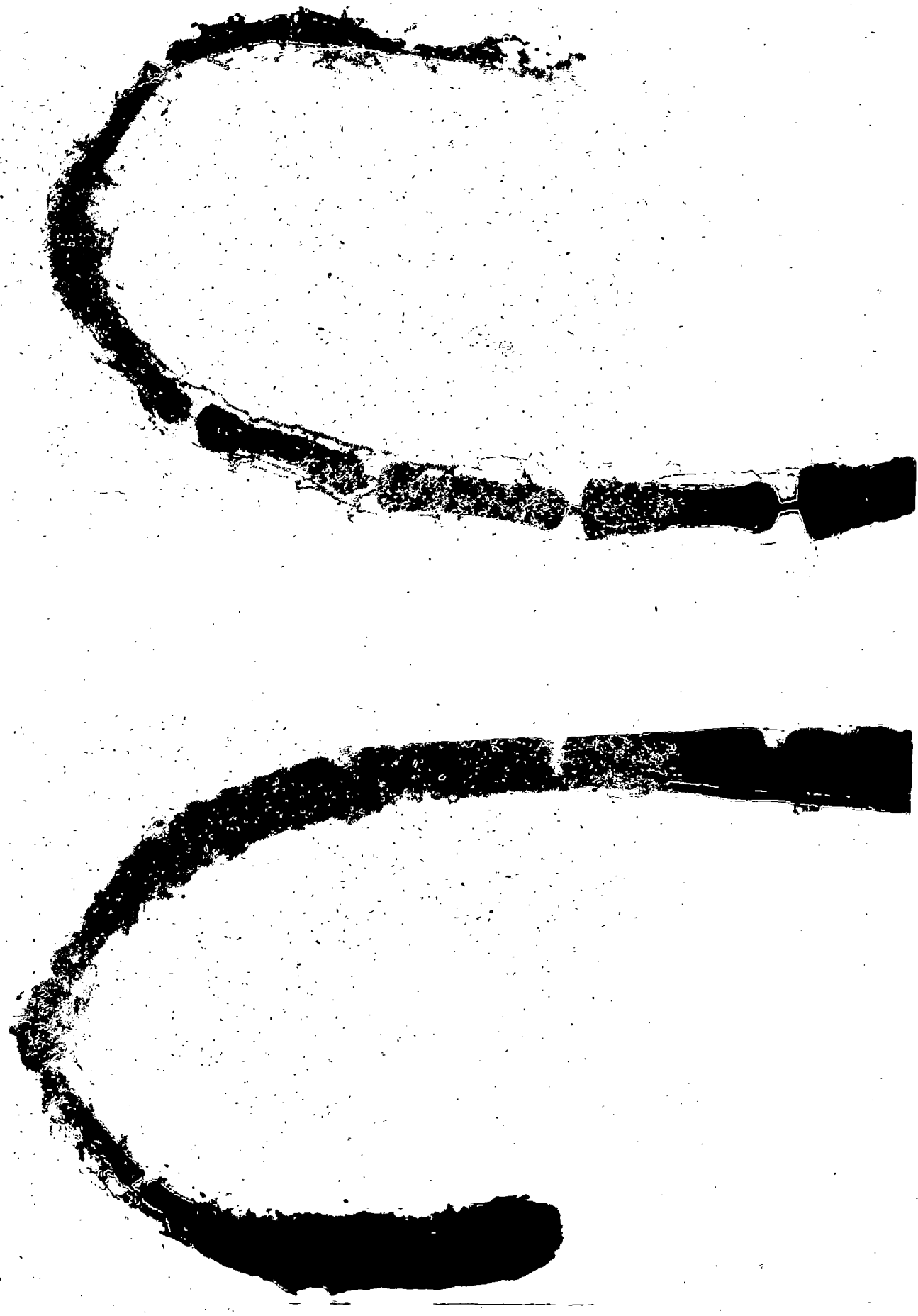


Fig. 3. Plain radiograph of diseased tail - osteolysis and separation of bone between healthy and necrosed segments. Necrosed portion shows demineralisation and hence a decreased density - An increase in the density of soft tissue at the level of necrosis.

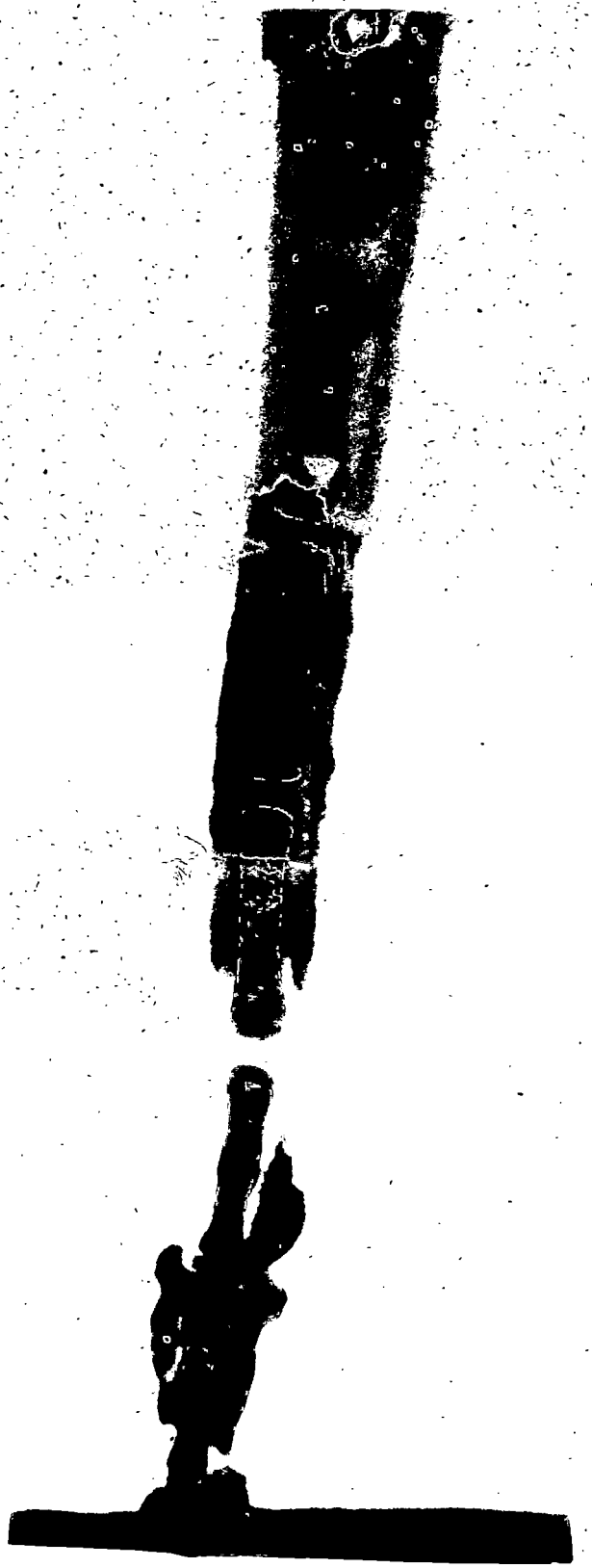


Fig. 4. Venogram of the diseased tail - commencement of venous capillaries in level with osteolytic portion and continuing with the lateral coccygeal veins.

Fig. 5. Arteriogram of the diseased tail - middle coccygeal artery shows increase in the number of arterioles towards the site of necrosis - Arterial structures terminate in level with the site of osteolysis.

Fig. 6. Tail necrosis - buffalo - Tail showing loss of hair, necrosis and scales over the tail

Fig. 7. Coccygeal artery - Hypertrophy of tunica media Van Gieson x 250

Fig. 8. Tail - Thickened vessels with various sizes of lumen H & E x 250

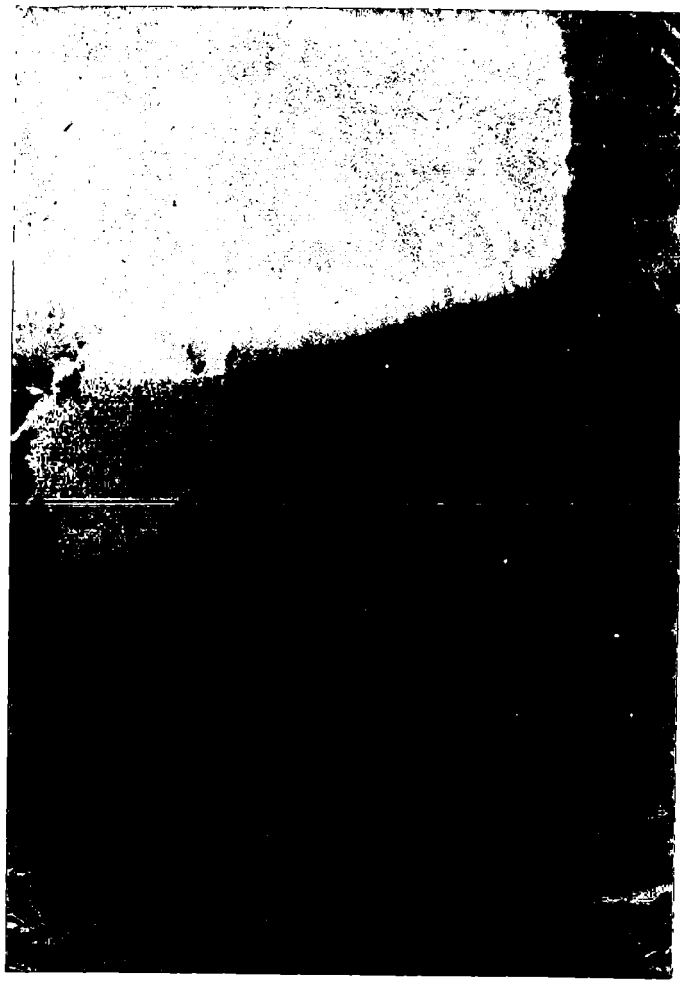


Fig. 9. Coccygeal artery - Thrombosis - varying stages of organisation of thrombosis.
H & E x 200

Fig. 10. Thrombosis - canalisation - blood vessels lined with endothelium.
H & E x 250

Fig. 11. Coccygeal artery - subintimal thrombosis.
Van Gieson x 250

Fig. 12. Coccygeal artery - fragmentation of elastic fibres.
Verhoeffs x 250

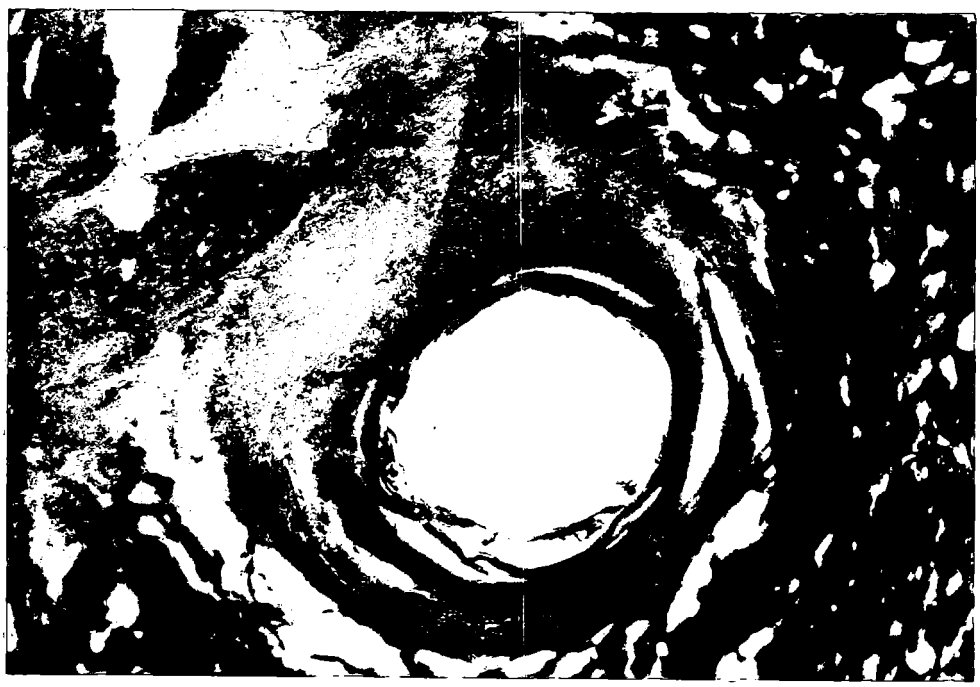


Fig. 13. Coccygeal artery - replacement fibrosis - proliferating spindle shaped fibroblasts seen.
Van Gieson x 250

Fig. 14. Granulation tissue - collaterals vessels around a thickened large blood vessel
H & E x 250

Fig. 15. Coccygeal artery - proliferation of pericytes Van Gieson x 250

Fig. 16. Coccygeal artery - fibrosis around the artery. Van Gieson x 250

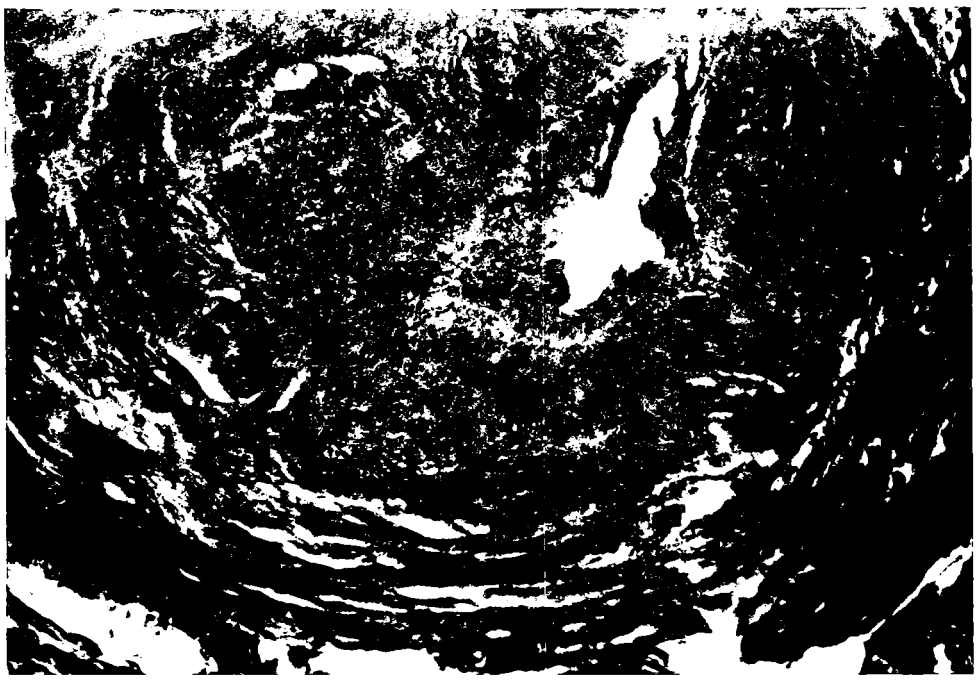
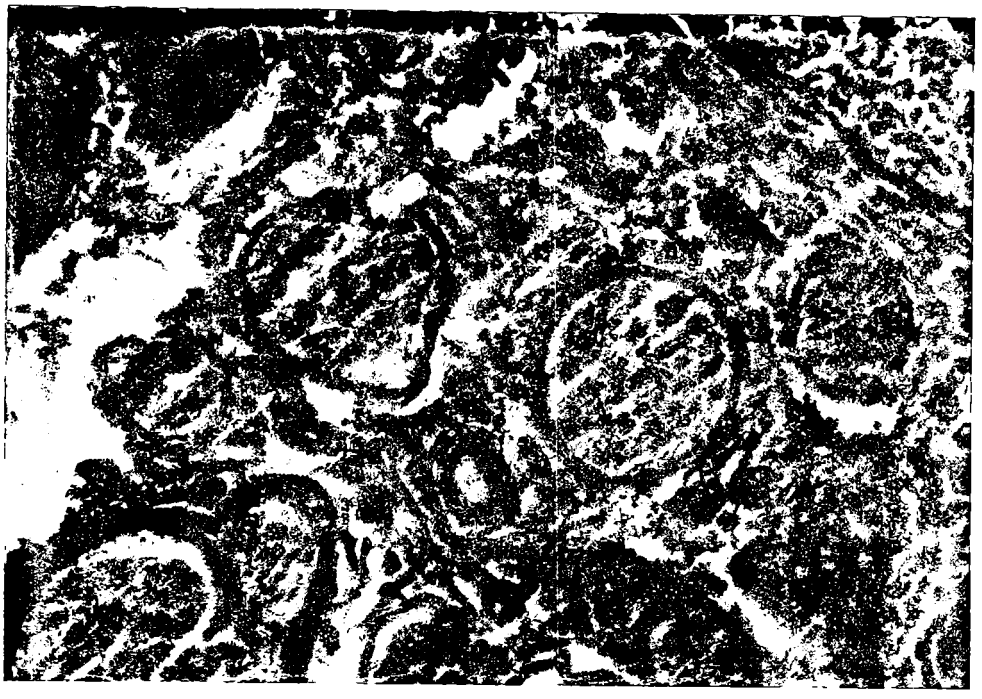


Fig. 17. Tail - Nerve fibres - seen scattered amidst a mass of fibro-collagenous tissue.
Van Gieson x 250

Fig. 18. Coccygeal nerve fibres - encircled by thick fibro-collagenous tissue
Van Gieson x 250



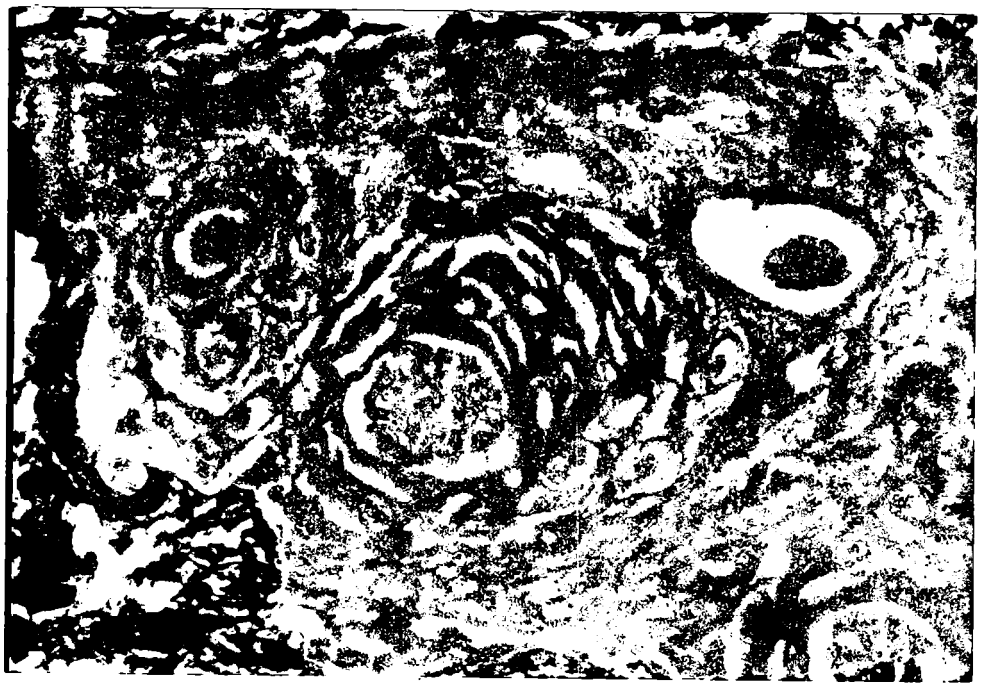
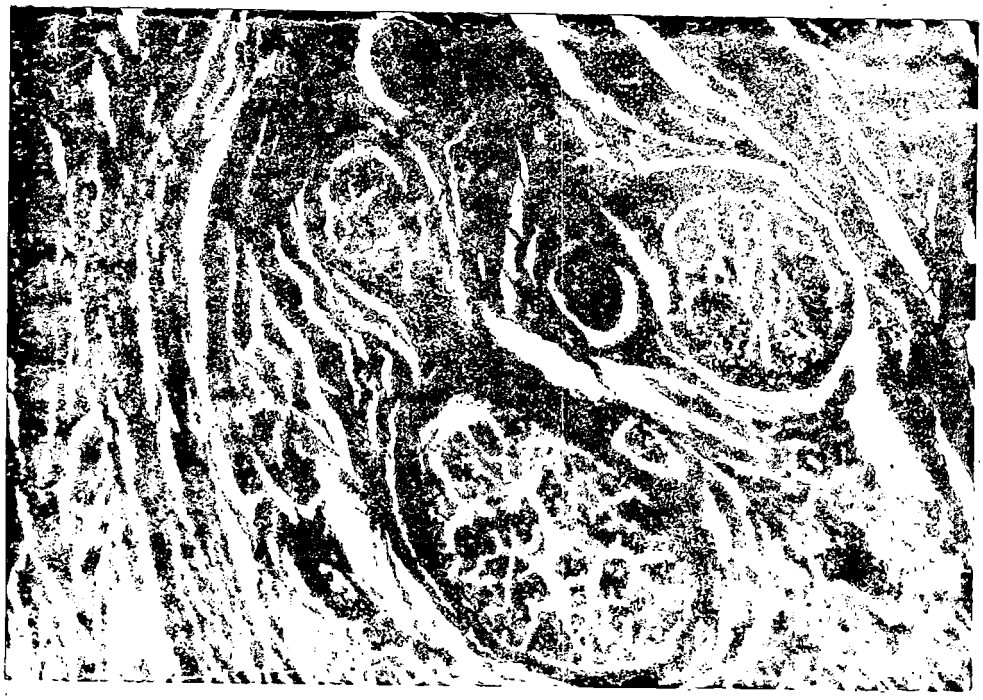


Fig. 19. Coccygeal nerve fibres - Intraneural and perineural fibrosis.
Van Gieson x 250

Fig. 20. Coccygeal vertebra - osteolysis - peripheral thinning of osseous tissue
Van Gieson x 250



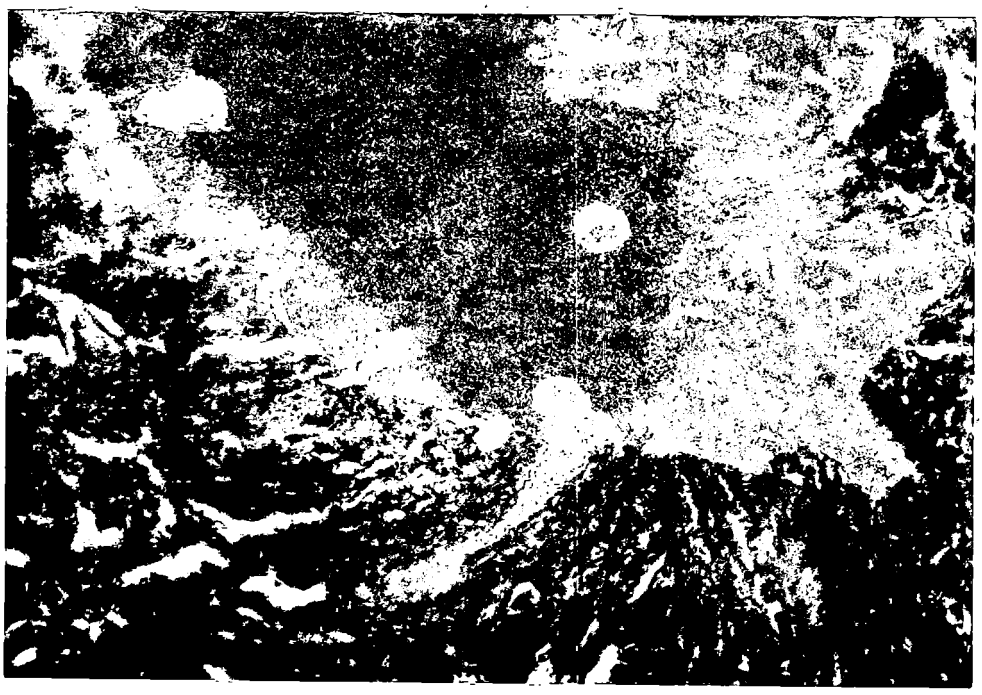


Fig. 21. Skin - tail - chronic dermetitis, hyperkeratosis, parakeratosis, moderate to severe acanthosis. H & E x 250

Fig. 22. Skin - tail - necrosis and oedema of the dermis. H & E x 400

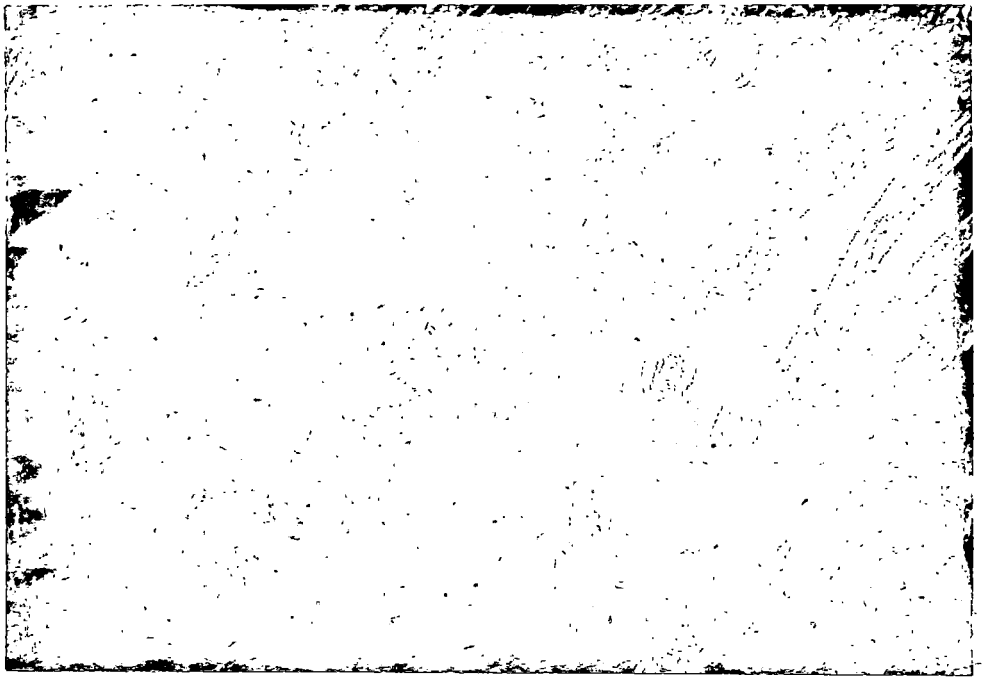
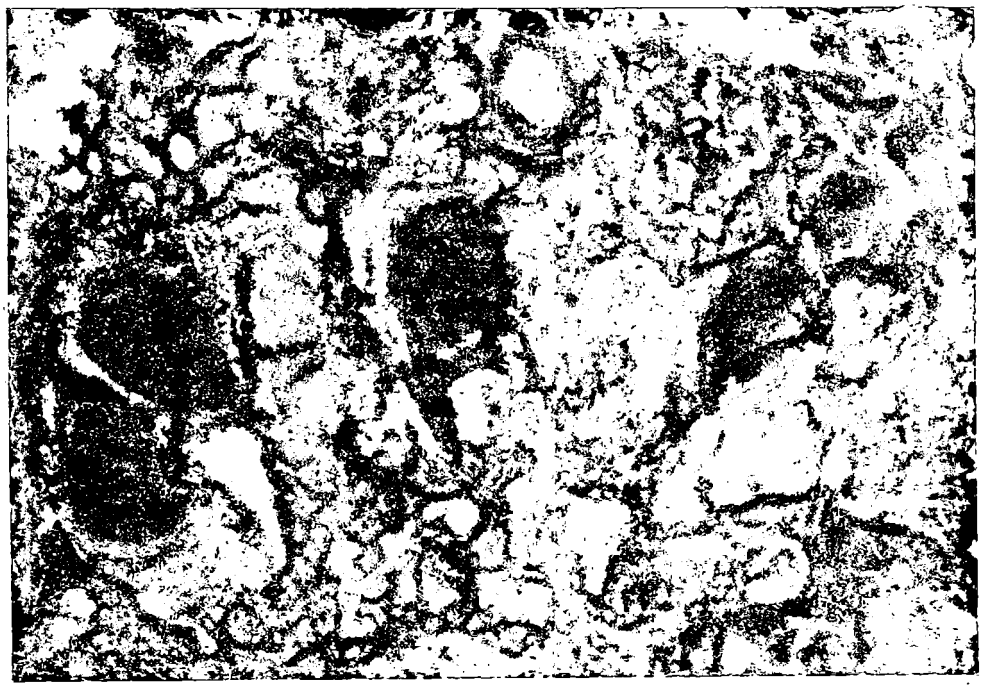


Fig. 23. Hair follicles - tail - degeneration of hair follicles
H & E x 250.

Fig. 24. Hair follicles - tail - cystic dilatation.
H & E x 250



Discussion

5. DISCUSSION

Necrosis of the extremities in cattle was prevalent in the State of Kerala since the three decades. However, no systematic documentation of the prevalence of the condition was undertaken. As per the data documented by the Disease Surveillence Unit of the Animal Husbandry Department the incidence was found to be more in buffaloes than in white cattle. This is an observation which would support the findings of the earlier workers on the prevalence of the condition (George et al. 1970). It appears that buffaloes are more prone to get the disease syndrome and the reason may be that buffaloes consume more paddy straw than white cattle and through this they may consume more amount of toxin. They may also be more sensitive to the effects of the toxins. The actual incidence must be more than this since many cases go unnoticed and reporting and documentation of disease occurrence are not practised.

The clinical features of the disease are very much apparent and a diagnosis of the disease is not a problem even at the farmers level. The absence of haematological changes and systemic disturbances would suggest that it is a slow degenerative disease. Estimation of blood parameters did not reveal any significant change and this is an observation which would suggest that there is no systemic disturbances as a result of the disease. As observed in the study the haemoglobin, erythrocyte sedimentation rate, packed cell

volume and differential count were reported to be within the normal range. Kwatra and Singh (1973) observed normal haematological values in experimental cases of Degnala disease. Bhatia and Kalra (1981) reported only slight gradual decline in the haematological parameters in animals suffering from severe form of Degnala disease.

Calcium, magnesium and inorganic phosphates were estimated in the serum and they were found to be within the normal range.

The plasma protein level was also found to be within normal range.

The radiographic studies of the necrosed tail revealed typical changes of aseptic separation of necrosed extremities. Plain radiographs showed the zone of necrosis well demarcated by a conical growth of granulation tissue around the vertebra at the affected region. The vertebra acted as the core around which the granulation tissue had developed. The coccigeal vertebra at the point of termination of granulation tissue had undergone lysis as evidenced by the radiotransparent zone in the body of the vertebra. The increased density of the granulation tissue was because of its increased vascularity. This was further clarified by the angiograms. The major artery terminated at level with the zone of necrosis and a large number of capillaries grew into the granulation tissue. The veins also had their origin in the granulation tissue indicating that the vascularity of the tissue had been

completely cut off by the necrosis and a new point of vascular communication had developed at the zone of necrosis. The radiographic studies undertaken have clearly demonstrated the vascular distribution in the necrosed part of the tail and it clarified that the thrombosis of the artery was the primary lesion. Unlike the usual bone reaction of proliferation along with degeneration in bone disorders, the affected vertebra showed only zonal lytic changes. This probably indicated that the entire vertebra had undergone primary necrosis and the growth of the granulation tissue had revasculated the bone with lytic separation taking place at the region where vascularity had not taken place.

The gross lesions observed were of mild type and they were confirmed to the tail. However, in the earlier reports by George et al. (1970) and Rajan et al. (1977) the lesions were described in the limbs, ears and mandibular and maxillary regions.

The histopathological studies carried out have precisely demonstrated the occlusive arterial lesions in the coccygeal arteries. The basic histological lesion appears to be hypertrophy of the muscular coat of the arterial walls. This was also described by Rajan et al. (1977). This perforce leads to the narrowing of the lumen and slowing of the blood flow. The morphological and functional alterations would no doubt lead to thrombosis. Thrombosis of varying magnitude was a consistent feature and this would support the proposed pathogenesis. Organisation, canalisation and calcification are

terminal pathological features seen in a thrombus. The thrombosis was specifically seen in the arteries, and the veins were exceptionally free or were less frequently involved. Absence of the thickening of the wall of the vein and consistent hypertrophy of the musculature of arteries suggest the possible involvement of the fungal toxins in the aetiopathogenesis of the condition. The haematological studies did not indicate any systemic disturbances and it would appear that the aetiological agent had a specific stimulatory effect on the arterial musculature. This again would support the muscular hypertrophic pathogenesis of the lesion. Osteolytic changes observed in sections were supported by radiographic studies. The collateral vascular anastomosis observed in the radiographs were seen in histopathological sections also. This is an observation which would again suggest a slow pathogenesis of the lesions. The involvement of coccygeal nerve appears to be a secondary manifestation of tissue ischaemia. The degenerative changes in the muscles, hair follicles and epidermis could be attributed to the disturbance in the circulation and consequent ischaemic tissue damage.

The blood vessels of the extremities being far away from the heart, enjoys less blood pressure (Woods et al. 1966) and hence the blood flow will be slower to these organs.

Trichothecenes are known to produce vasoconstriction (Wilson and Gentry, 1985). It may be hypothesised that the

mycotoxins circulating in small quantities in blood may have prolonged effect on the arteries of the extremities and it may cause persistent vasoconstriction. Normally vasoconstriction is a transient phenomenon, resulting in narrowing of the lumen. But when the agent is persisting, continuous muscular contractions of tunica media may lead to muscular hypertrophy. The slowing of the blood flow favours quick thrombosis, when there is narrowing of the lumen of the vessel following the hypertrophy of the arterial musculature.

Investigations were undertaken to identify the aetiology of the condition. The background history provided by the farmers suggested paddy straw as the incriminating agent. The history that the affected animals were solely fed on paddy straw and they were damaged ones supported the conclusion that the damaged paddy straw was the causative agent. Eaerlier workers (Kousuri et al. 1970; Tookey et al. 1972; Kalra et al. 1977; Maqsood, 1984; Behera, 1985; Deng et al. 1985; Zhang et al. 1985; Bhatia and Gupta, 1986) had also incriminated paddy straw infected with Fusarium species of fungi as the causative agent. Fusarium species of fungi produce Trichothecene toxins having a tetracyclic 12, 13, epoxy - A^9-trichothecene skeleton. These toxins have no unique properties that provide easy identification. Pure compounds are colourless and have no fluorescent properties or absorption bands under U.V. or visible light (Ciegler et al. 1983; Rao et al. 1985). Despite these unfavourable properties, thin layer chromatography on

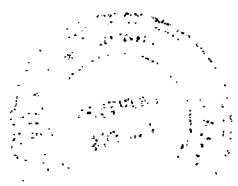
silica gel is the most convenient and most used procedure for identification of trichothecenes, when the toxins are in high concentration and interferring substances are not abundant. In the present study, using thin layer chromatography method, no toxins could be detected from the field samples collected. Probably the amount of toxins would have been too low to be detected by the method. A major factor which was beyond the control was the fluctuation in the source of the paddy straw. Unlike the north Indian farmer, the marginal and small farmers of Kerala are not in a position to provide their livestock with paddy straw from a single source purchased in bulk. When the places were visited for investigation it was informed by farmers that straw was purchased in small quantities. Hence the original sample of straw, responsible for the condition might have been used up. Kalra et al. (1977) reported that they were not able to detect any toxin from the experimentally inoculated straw with Fusarium species of fungi. Bhatia and Gupta (1986) observed that Fusarium toxin had specific role in the pathogenesis of the disease condition. In the present study with field samples of paddy straw Fusarium fungi could not be identified. Behera (1985) experimentally produced Degnala disease in buffaloes by feeding paddy straw contaminated with T-2 toxins. He concluded that the Degnala disease is caused by ingestion of paddy straw contaminated with Trichothecenes.

Arora et al. (1975) observed that the selenium content in the paddy straw was responsible for this condition. The

pathological investigation undertaken in this study did not reveal a sequence of tissue changes attributable to selenosis. Jayakumar (1989) undertook an experimental study on selenium toxicosis and could not reproduce the condition and pointed out that selenium toxicity is not the causative factor of this disease syndrome.

Although in the present study Fusarium species of fungi could not be isolated and Trichothecenes could not be identified, the pathological features observed and the clinical course manifested suggested a mycotoxic aetiology. There is need to undertake further investigation to confirm the role of Trichothecenes in the causation of this disease syndrome.

Summary



6. SUMMARY

The study on the prevalence and pathology of necrosis of extremities in cattle was undertaken at the Centre of Excellence in Pathology, College of Veterinary and Animal Sciences in liaison with University Veterinary Hospitals as well as the institutions under the State Animal Husbandry Department. The prevalence of the disease was found to be more in buffaloes than in white cattle.

The chief clinical manifestation was the necrosis of the extremity of the tail. Investigations revealed that the condition was associated with the ingestion of mouldy paddy straw. Animals were found to be free from any systemic involvement.

Mean Haemoglobin, Packed cell volume, Erythrocyte sedimentation rate, Total Count and Differential Count were estimated in the affected animals. The data did not reveal any significant change from the normal.

Calcium, Magnesium and Inorganic Phosphate levels in the serum of the affected animals were found to be within the normal range.

The plasma protein level also did not reveal any significant change.

The origin, distribution and features of the vascular channels in the disease syndrome were demonstrated for the first time by radiographic studies. There was an increase in the density of soft tissue at the zone of necrosis with

lytic changes in coccygeal vertebra at the level of necrosis. Arteriogram of the diseased tail showed that the middle coccygeal artery terminated at level with the vertebra preceding the one which had undergone lysis. A large number of tortous arterial branches originated from the artery and they supplied the soft tissue at the zone of necrosis. Venograms showed that the lateral coccygeal veins originated from the level of the diseased vertebra. Arteriovenous anasthamosis was very much appreciated.

It was indicated by systematic histopathological studies that the vascular lesions were the basis of the pathogenesis of the condition. Partial or complete thrombosis with organisation and canalisation were seen in the arteries. The most conspicuous lesion was the hypertrophy of the tunica media and consequent narrowing of the lumen. Veins were free from such lesions. Collateral neovascularisation was evident.

Coccygeal vertebral osteolysis, degeneration of nerve fibres, perineural fibrosis, muscular hyalinization, chronic dermetitis, hyperkeratosis, parakeratosis, degeneration and hyalinization of hair follicles were observed. They were considered to be secondary changes following arterial thrombosis.

Samples of paddy straw collected from 14 different places in various districts were screened for the fungal flora. Aspergillus, Rhizopus, Penicillium, Mucour, Alternaria, Curvularia and Nigrospora were identified. Samples of paddy straw were screened for trichothecenes using Thin Layer Chromatography. Trichothecenes could not be detected.

Eventhough Fusarium species of fungi could not be identified and trichothecenes could not be detected, the close association of the condition with the feeding of contaminated paddy straw, and pathological features observed suggest a mycotoxic aetiology. Further studies are needed to confirm the role of trichothecenes in the causation of this disease.

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PREVALENCE AND PATHOLOGY OF NECROSIS OF EXTREMITIES IN CATTLE

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ABSTRACT OF A THESIS

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ABSTRACT

A study on the prevalence and pathology of necrosis of extremities in cattle was conducted. It was found that the disease was more prevalent in buffaloes than in white cattle. The chief clinical manifestation was necrosis of tail. A close association between mouldy paddy straw and the disease syndrome was observed. Animals observed during the study were found to be free from systemic disturbances.

The mean Haemoglobin, Packed Cell Volume, Erythrocyte Sedimentation Rate, Total count and Differential count were estimated. The data did not reveal any significant change in affected animals. Calcium, Magnesium and inorganic phosphate levels in the serum of affected animals were found to be normal. Total plasma protein level also did not show any significant variation.

The origin, distribution and features of the vascular channels in this disease syndrome were demonstrated for the first time by radiographic studies. Plain radiograph of the diseased tail revealed an increase in the density of the soft tissue at the zone of necrosis with lytic changes in the coccygeal vertebra at the level of necrosis. Arteriogram of the diseased tail revealed that the middle coccygeal artery terminated at level with the vertebra preceding the one which had undergone lysis. A large number of tortous arterial branches originated from the artery and they supplied the soft tissue at the zone of necrosis. Venogram showed that

the lateral coccygeal veins originated from the level of the diseased vertebra. Arteriovenous anastomosis was appreciated.

Histopathological studies undertaken on the necrosed tails revealed that the vascular lesions were the basis for the pathogenesis of the condition. The most conspicuous lesion was the hypertrophy of tunica media of the coccygeal arteries and consequent narrowing of the lumen. Partial or complete thrombosis with organisation and canalisation were observed in the arteries. Veins were found to be free from such lesions. Collateral neovascularisation was evident. Coccygeal vertebral osteolysis was observed. Radiographic studies have supported these findings. Perineural fibrosis, degeneration of nerve fibres, muscular hyalinization, were also noticed. Chronic dermetitis, hyperkeratosis, parakeratosis, acanthosis, degeneration and hyalinization of hair follicles were observed in the skin.

Samples of paddy straw collected from 14 different places in various districts were the disease had occurred were screened for fungal flora. Aspergillous, Rhizopus, Penicillium, Mucour, Alternaria, Curvularia and Nigrospora were identified. Samples were screened for trichothecenes using Thin Layer Chromatography. Trichothecenes could not be detected.

Eventhough Fusarium species of fungi could not be identified and trichothecenes could not be detected, the close association of the condition with feeding of mouldy paddy straw and pathological features observed suggest a mycotoxic aetiology. Further studies are needed to confirm the role of trichothecenes in the causation of this disease.